Biol Blood Marrow Transplant 21 (2015) 920–925

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

ASBMT American Society for Blood and Marrow Transplantation

Clinical Research: Alternative Donors

Intensified Mycophenolate Mofetil Dosing and Higher Mycophenolic Acid Trough Levels Reduce Severe Acute Graft-versus-Host Disease after Double-Unit Cord Blood Transplantation

Stephen Harnicar [1,2,*], Doris M. Ponce [1,3], Patrick Hilden [4], Junting Zheng [4], Sean M. Devlin [4], Marissa Lubin [1], Melissa Pozotrigo [2,5], Sherry Mathew [2], Nelly Adel [2], Nancy A. Kernan [5], Richard O'Reilly [5], Susan Prockop [5], Andromachi Scaradavou [5], Alan Hanash [1,3], Robert Jenq [1,3], Marcel van den Brink [1,3], Sergio Giralt [1,3], Miguel A. Perales [1,3], James W. Young [1,3], Juliet N. Barker [1,3]

[1] *Adult Bone Marrow Transplantation Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York*
[2] *Department of Pharmacy, Memorial Sloan Kettering Cancer Center, New York, New York*
[3] *Department of Medicine, Weill Cornell Medical College, New York, New York*
[4] *Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, New York*
[5] *Bone Marrow Transplantation Service, Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, New York*

Article history:
Received 6 November 2014
Accepted 28 January 2015

Key Words:
Cord blood transplantation
Graft-versus-host disease
Mycophenolate mofetil
Pharmacokinetics

A B S T R A C T

Although mycophenolate mofetil (MMF) has replaced corticosteroids as immunosuppression in cord blood transplantation (CBT), optimal MMF dosing has yet to be established. We intensified MMF dosing from every 12 to every 8 hours to augment graft-versus-host disease (GVHD) prophylaxis in double-unit cord blood transplantation (dCBT) and evaluated outcomes according to the total daily MMF dose/kg in 174 dCBT recipients (median age, 39 years; range, 1 to 71) who underwent transplantation for hematologic malignancies. Recipients of an MMF dose $\leq$ the median (36 mg/kg/day) had an increased day 100 grade III and IV acute GVHD (aGVHD) incidence compared with patients who received >36 mg/kg/day (24% versus 8%, $P = .008$). Recipients of $\leq$ the median dose who had highly HLA allele (1 to 3 of 6) mismatched dominant units had the highest day 100 grade III and IV aGVHD incidence of 37% ($P = .009$). This finding was confirmed in multivariate analysis ($P = .053$). In 83 patients evaluated for mycophenolic acid (MPA) troughs, those with a mean week 1 and 2 trough < .5 μg/mL had an increased day 100 grade III and IV aGVHD of 26% versus 9% ($P = .063$), and those who received a low total daily MMF dose and had a low mean week 1 and 2 MPA trough had a 40% incidence ($P = .008$). Higher MMF dosing or MPA troughs had no impact on engraftment after myeloablation. This analysis supports intensified MMF dosing in milligram per kilogram per day and MPA trough level monitoring early after transplantation in dCBT recipients.

INTRODUCTION

Mycophenolate mofetil (MMF) is an inactive morpholinoethyl ester prodrug that inhibits the proliferation of T and B lymphocytes and is designed to enhance the bioavailability of the active form, mycophenolic acid (MPA). MMF has been a successful immunosuppressive agent in adult donor allogeneic hematopoietic stem cell transplantation [1]. Consequently, MMF was introduced in unrelated donor cord blood (CB) transplantation (CBT) to avoid the multiple toxicities of corticosteroids [2]. However, some centers have had concerns that MMF could be associated with myelosuppression in CBT recipients [3,4]. Furthermore, MPA's pharmacokinetics (PK) are characterized by a high intra- and inter- patient variability [5,6], and inadequate MPA levels have been correlated with a reduced efficacy of prophylaxis and treatment of acute graft-versus-host disease (aGVHD) [7,8].

To augment MMF exposure, MMF has been increased from 1 g every 12 hours to 1 g every 8 hours in adult allograft recipients [9-11]. As aGVHD is a leading cause of morbidity

Financial disclosure: See Acknowledgments on page 925.

* Correspondence and reprint requests: Stephen Harnicar, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065.
E-mail address: harnicas@mskcc.org (S. Harnicar).

http://dx.doi.org/10.1016/j.bbmt.2015.01.024
1083-8791/

and transplantation-related mortality (TRM) in CBT [12-14], intensified MMF dosing has also been adopted in CBT [15]. Dosing in patients ≥50 kg, however, has not taken into account the recipient's weight. Hence, the association between MMF dosing and both aGVHD prevention and the potential toxicities to the bone marrow and gastrointestinal tract merit investigation [16]. As there is also no established target MPA exposure for CBT recipients, the association between MPA PK parameters and these endpoints also require evaluation. We have, therefore, investigated the transplantation outcomes in 174 double-unit CBT (dCBT) recipients treated for hematologic malignancies according to the total MMF dose in milligrams per kilogram per day. We hypothesized that MMF doses above the median total daily dose would be associated with a reduced incidence of grade III and IV aGVHD without an adverse effect upon neutrophil engraftment. In a subset of patients, the association between total MPA trough levels and day 100 aGVHD and engraftment was also evaluated.

METHODS

Patients and Graft Characteristics

Patients underwent transplantation at Memorial Sloan Kettering Cancer Center (MSKCC) between October 1, 2005 and May 31, 2013. Patients eligible for this analysis included all consecutive adult and pediatric recipients of first allografts who underwent dCBT for the treatment of hematologic malignancies with the exclusion of acute leukemia patients who underwent transplantation with ≥20% bone marrow blasts. All patients received double-unit CB grafts per institutional standard practice. CB units were selected on the basis of 4 to 6 of 6 HLA-A, -B antigen, -DRB1 allele match to the recipient, a cryopreserved total nucleated cell dose of at least 1.5×10^7/kg/unit, and the bank of origin, as previously described [17]. Unit-unit HLA match was not considered. High resolution HLA-A, -B, -C, -DRB1, and -DQ allele typing of CB units was performed routinely for research purposes and usually did not influence unit selection. All patients provided written informed consent for transplantation and transplantation outcome analysis on protocols approved by the MSKCC Institutional Review and Privacy Board.

Conditioning and GVHD Prophylaxis

Pretransplantation conditioning varied according to the patient's age, diagnosis, remission status, extent of prior therapy, and comorbidities, and consisted of high-dose, reduced-intensity (but functionally myeloablative), and nonmyeloablative regimens as previously described [13,18]. Intravenous calcineurin inhibitors (CNI), predominantly cyclosporine A (CSA), and MMF comprised GVHD prophylaxis starting on day -3 intravenously with no antithymocyte globulin. CSA was dosed to achieve a trough level 200 to 400 ng/mL and tacrolimus was dosed to achieve trough levels of 5 to 12 ng/mL. Before September 2009, 81 patients (47%) received MMF 1 g intravenously every 12 hours (those ≤12 years or <50 kg received 15 mg/kg/dose). From September 2009, intensified MMF dosing was instituted to augment GVHD prophylaxis. Ninety-three patients (53%) received MMF at 1 g intravenously every 8 hours for patients both >12 years and ≥50 kg (or 15 mg/kg/dose if >12 years but <50 kg). Patients ≤12 years received 20 mg/kg/dose. Granulocyte colony–stimulating factor (5 μg/kg/day rounded to vial size) was given after CBT to all patients until neutrophil recovery.

Standard practice to taper MMF was to reduce the dose at approximately 75 to 100 days after transplantation if there was no aGVHD. In patients dosed every 8 hours, this interval was maintained for as long as possible unless there were adherence problems with this schedule, in which case a switch to twice daily dosing was made. The taper was performed at 10% to 25% decrements in all patients with the goal of being off MMF by 4 to 6 months; however, this schedule was ultimately at the physician's discretion. For example, increased relapse risk may have resulted in a hastened taper schedule with attention to the development of aGVHD symptoms. The most frequent reason to delay the MMF taper other than aGVHD was significant renal impairment preventing therapeutic cyclosporine levels. Dosing modifications were very rarely made for delayed engraftment early after transplantation. However, later in the transplantation course, dosing reductions were sometimes done for cytopenias usually in the context of concurrent valganciclovir therapy.

Sample Collection and Analysis for MPA Trough Levels

Serial weekly total MPA trough levels were collected on days 1, 8, 15, 22, 29, and 36 after dCBT from a subset of 85 patients who underwent transplantation from August 1, 2009 to November 30, 2012. Troughs were evaluated because of the substantial logistical advantage over the serial blood samples required for area under the curve measurements. Samples were collected primarily for research purposes. However, in 8 of 85 (9%) patients, dosing was increased to every 8 hours in week 2 as a result of week 1 trough results. Samples of ≥1 mL serum were collected in a no additive red top tube. Peripheral blood was collected in adults, whereas the sample in children was drawn from a non-MMF–infused line. The blood was allowed to clot for 20 to 30 minutes at 15°C to 28°C, centrifuged at 2700 rpm for 10 minutes, and sent for analysis by liquid chromatography mass spectrometry methodology at Quest Diagnostics Laboratories, Teterboro, NJ. The result was available within 3 to 4 days.

Study Definitions

Neutrophil recovery was defined as the first of 3 consecutive days with an absolute neutrophil count $\geq .5 \times 10^9$/L, whereas *platelet recovery* was the first of 3 consecutive days $\geq 20 \times 10^9$/L and at least 7 days without transfusion support. *Engraftment* was defined as sustained donor-derived count recovery with donor chimerism > 90% (both units combined). aGVHD was diagnosed clinically with histologic confirmation when appropriate. The grade of aGVHD was based on the International Bone Marrow Transplant Registry classification [19], except grades A to D were labeled grades I to IV. Grading was reviewed by a transplantation clinician panel to reach a consensus of maximum aGVHD grade. *Chronic GVHD* was defined according to the National Institutes of Health consensus criteria [20]. *Relapse* was defined as recurrence or progression of disease over pretransplantation baseline, and TRM was death from any cause in continued remission. *Progression-free survival* (PFS) was defined according to standard criteria.

Statistical Analysis

For analysis purposes, patients were a priori divided into 2 dosing groups: above or below the median total daily milligram per kilogram MMF dose. The strategy to analyze outcomes according to above versus below the median total daily dose was chosen, as deriving a threshold based on the observed data would require a separate validation cohort. Descriptive analyses were performed for patient demographics and differences across low versus high MMF groups and compared using the Wilcoxon rank-sum test for continuous covariates. Chi-square and Fisher's exact test were used for categorical covariates as indicated. The cumulative incidence method for competing risks was used to calculate the cumulative incidence of engraftment, GVHD, TRM, and relapse. Gray's test was used to compare the incidence of select outcomes across MMF dose levels. PFS was calculated using Kaplan-Meier methodology and compared across MMF dose levels using a log-rank test.

In the subset analyses of MPA trough levels, descriptive statistics summarized the changes in MPA trough levels over the first 6 weeks after transplantation and the trends across age and conditioning regimens. Fisher's exact test evaluated associations between patient clinical factors and mean week 1 and 2 trough levels dichotomized at .5 μg/mL. To evaluate the association between MPA trough levels and dCBT outcomes, mean levels of week 1 and week 2 combined were dichotomized at <.5 μg/mL and ≥.5 μg/mL for efficacy and <2 μg/mL and ≥2 μg/mL for toxicity. Starting from a landmark of 2 weeks after transplantation, cumulative incidence functions and Gray's test were used to estimate and compare engraftment and aGVHD by mean MPA trough levels. All analyses were conducted using R statistical software, version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Comparison of Patient Characteristics According to MMF Dosing

One hundred seventy-four patients were analyzed. The majority (n = 136, 78%) received myeloablative conditioning. The median total daily dose of MMF was 36 mg/kg. Patients who received low (below the median) and high (above the median) total daily doses were comparable in terms of age, recipient cytomegalovirus (CMV) status, disease type and risk, conditioning regimen intensity, and graft characteristics (dominant or engrafting unit infused $CD34^+$ cell dose and 6 HLA-allele match) (Table 1). However, patients in the low dosing group were more likely to be male, heavier, and receive MMF every 12 hours.

Association between MMF Dosing and aGVHD

Transplantation outcomes according to MMF dosing below versus above the median total daily MMF dose/kg are shown in Table 2. There was no difference in the cumulative

Table 1
Patient and Graft Demographics according to Total Daily MMF Dose per Kilogram*

Characteristic	mTDD ≤ 36 (n = 101)	mTDD > 36 (n = 73)	*P* Value
Age, median (range), yr	42 (1-66)	36 (2-71)	.181
Recipient gender, n (%)			
Male	70 (69%)	27 (37%)	<.001
Female	31 (31%)	46 (63%)	
Weight, median (range), kg			
Overall	80 (7-125)	54 (10-77)	<.001
Male	82 (7-125)	63 (11-76)	<.001
Female	70 (13-104)	53 (10-77)	<.001
Recipient CMV serostatus, n (%)			
Negative	51 (51%)	27 (37%)	.107
Positive	50 (50%)	46 (63%)	
Disease type, n (%)			
Acute leukemia	52 (52%)	48 (66%)	.173
MDS/CML/other MPD	7 (7%)	3 (4%)	
NHL/HL/CLL	42 (42%)	22 (30%)	
Disease risk, n (%)			
Standard	11 (11%)	9 (12%)	.958
High	90 (89%)	64 (88%)	
Conditioning intensity, n (%)			
Myeloablative	78 (77%)	58 (80%)	.869
Nonmyeloablative	23 (23%)	15 (21%)	
Dosing interval, n (%)			
Every 12 h	71 (70%)	10 (14%)	<.001
Every 8 h	30 (30%)	63 (86%)	
Dominant unit median infused $CD34^+$ cell dose (range), $\times 10^5$/kg			
Larger unit	1.30 (.26-6.42)	1.24 (.41-4.77)	.793
Smaller unit	.68 (.13-1.52)	.66 (.08-2.12)	.976
Unit 6 allele HLA match, n (%)†			
1-3/6	75 (37%)	42 (29%)	.130
4-6/6	127 (63%)	104 (71%)	

mTDD indicates median total daily dose; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; MPD, myeloproliferative disorder; NHL, non-Hodgkin's lymphoma; HL, Hodgkin's lymphoma; CLL, chronic lymphocytic leukemia.

* Below versus above the median total daily dose in milligram per kilogram.

† The HLA match grade reflects 202 units in the low MMF patient group and 146 units in the high group.

incidences of grade II to IV aGVHD at day 100 in the low and high dosing groups (53% versus 47%, $P = .345$). The incidence of severe (grade III and IV) aGVHD at day 100, however, was 3-fold higher in recipients of a total daily MMF dose below the median versus those in the high group (24% versus 8%, $P = .008$). One-year chronic GHVD was not different in the 2 groups (12% versus 11%, $P = .422$).

In univariate analysis of variables potentially associated with the incidence of day 100 grade II to IV aGHVD, aGVHD was associated with gender (57% in male versus 42% in female patients, $P = .027$) and CMV serostatus (58% in seronegative versus 44% in seropositive dCBT recipients, $P = .032$). The results of univariate analysis of grade III and IV aGVHD are shown in Table 3. Male gender was associated with a higher incidence of severe aGVHD (25% versus 8%, $P = .003$). Despite males being heavier, recipient weight (below or above the median) was not significant, and analyzing the patient's weight as a continuous variable or in quartiles also showed no differences in aGVHD incidence. The association between male gender and grade III-IV aGVHD persisted in both patients whose weight was below the median or in the subset whose weight was above the median. Additionally, among male patients only, there was no association between weight and grade III-IV aGVHD (data not shown). Finally, in male patients there was no difference in the gender of the dominant unit and, therefore, gender mismatch did not explain increased aGVHD incidence in males.

Table 2
dCBT Outcomes by Total Daily MMF Dose per Kilogram*

Variable	95% CI	*P* Value
Day 45 neutrophil engraftment†		
Low MMF dose (n = 78)	95% (86-98)	.990
High MMF dose (n = 58)	95% (83-99)	
Day 100 aGVHD II-IV		
Low MMF dose (n = 101)	53% (42-62)	.345
High MMF dose (n = 73)	47% (35-58)	
Day 100 aGVHD III-IV		
Low MMF dose (n = 101)	24% (16-33)	.008
High MMF dose (n = 73)	8% (3-16)	
1-yr chronic GVHD		
Low MMF dose (n = 101)	12% (7-19)	.422
High MMF dose (n = 73)	11% (5-20)	
Day 180 TRM		
Low MMF dose (n = 101)	20% (13-28)	.371
High MMF dose (n = 73)	21% (12-31)	
1-yr relapse		
Low MMF dose (n = 101)	14% (8-21)	.518
High MMF dose (n = 73)	15% (8-24)	
1-yr PFS		
Low MMF dose (n = 101)	63% (53-72)	.225
High MMF dose (n = 73)	59% (47-69)	

* Below versus above the median.

† Myeloablative CBT recipients only.

In univariate analysis, there was also a significant association between grade III and IV aGVHD and the combination of MMF dose and dominant unit-recipient HLA match ($P = .009$, Table 3). Recipients of total daily MMF doses below the median who had dominant units with a high degree of HLA mismatch (n = 30) had the highest incidence of day 100 grade III and IV aGVHD at 37%. In contrast, patients with a

Table 3
Univariate Analysis of Day 100 Grade III-IV aGVHD according to Patient and Graft Characteristics

Variable	Cumulative Incidence (95% CI)	*P* Value
Age		
0-15 yr (n = 35)	26% (13-41)	.129
≥16 yr (n = 139)	15% (10-22)	
Recipient gender		
Male (n = 97)	25% (17-34)	.003
Female (n = 77)	8% (3-15)	
Engrafting unit gender in males*		
Male (n = 54)	26% (15-38)	.478
Female (n = 40)	20% (9-34)	
Median weight, kg		
≤67.5 (n = 87)	17% (10-26)	.973
>67.5 (n = 87)	17% (10-26)	
Recipient CMV serostatus		
Negative (n = 78)	24% (16-34)	.026
Positive (n = 96)	12% (6-19)	
Conditioning intensity		
Myeloablative (n = 136)	18% (12-25)	.800
Nonmyeloablative (n = 38)	16% (6-29)	
Median MMF dose (mg/kg/d)		
≤36 (n = 101)	24% (16-33)	.008
>36 (n = 73)	8% (3-16)	
Dominant unit-recipient 6-allele HLA Match		
1-3/6 (n = 48)	25% (14-38)	.105
4-6/6 (n = 126)	14% (9-21)	
Median total daily MMF dose and dominant unit-recipient 6 allele HLA match		
Low (≤36) and worse (1-3/6) (n = 30)	37% (20-54)	.009
High (>36) and worse (1-3/6) (n = 18)	6% (0-23)	
Low (≤36) and better (4-6/6) (n = 71)	18% (10-28)	
High (>36) and better (4-6/6) (n = 55)	9% (3-19)	

* Dominant unit gender unknown in 3 patients.

Table 4
Multivariate Analysis of Variables Potentially Associated with Day 100 Grade III-IV aGVHD Risk

Variable	HR (95% CI)	*P* Value
Age, yr		
0-15 (n = 35)	Reference	.217
≥16 (n = 139)	.58 (.24-1.38)	
Recipient gender		
Male (n = 97)	Reference	.041
Female (n = 77)	.38 (.15-.96)	
Recipient CMV serostatus		
Negative (n = 78)	Reference	.190
Positive (n = 96)	.58 (.26-1.31)	
Median total daily MMF dose and dominant unit-recipient 6-allele HLA match		
Low (≤36) and worse (1-3/6) (n = 30)	Reference	.053
High (>36) and worse (1-3/6) (n = 18)	.23 (.03-1.84)	
Low (≤36) and better (4-6/6) (n = 71)	.46 (.20-1.07)	
High (>36) and better (4-6/6) (n = 55)	.26 (.09-.75)	

high MMF dose had an incidence of severe aGVHD of <10%. Severe aGVHD incidence in those with a low MMF dose but better HLA allele–matched engrafting unit was intermediate at 18%. Differences in severe aGVHD by dominant unit-recipient HLA match alone did not reach significance.

In a multivariate analysis that included age, gender, CMV serostatus, and the 4 category combination of MMF dose and HLA mismatch, males were significantly more likely to develop grade III and IV aGVHD ($P = .041$) (Table 4). The risk of grade III and IV aGVHD also differed by MMF dose and degree of HLA mismatch combination, with the highest risk in those who received a low MMF dose and higher HLA mismatch ($P = .053$).

Association between MMF Dosing and Engraftment and Survival Endpoints

Given that most nonmyeloablative dCBT recipients had transient autologous recovery, the effect of MMF dosing on donor engraftment was only compared among myeloablated dCBT recipients. The cumulative incidences of day 45 neutrophil engraftment were nearly identical regardless of MMF dosing, with each being 95% in the low and high MMF dosing groups at medians of 23 and 24 days, respectively ($P = .990$) (Table 2). The cumulative incidences of day 180 platelet engraftment were also similar: 82% (95% confidence interval [CI], 71% to 89%; median, 49 days; range, 29 to 162) in the low dose group and 86% (95% CI, 72% to 94%; median, 46 days; range, 30 to 137) in the high dose group ($P = .270$).

There were no significant differences in the cumulative incidences of TRM or relapse by dosing group, and the PFS were also similar (Table 2). Of 49 TRM deaths, GVHD was the most common cause (n = 22) followed by organ toxicity (n = 13). Infection as the primary cause of death was uncommon, occurring in only 7 patients overall. There were no differences in the percentage of patients dying of GVHD in low versus high MMF dosing groups. Eleven of 22 patients who died of GVHD did so before day 180. Of these 22 patients, aGVHD onset was before day 100 in all but 1 patient, and all 22 patients who died of GVHD had either aGVHD (n = 20) or aGVHD followed by overlap syndrome (n = 2).

Subset Analysis of Total MPA Trough Levels

Serial MPA troughs during weeks 1 to 6 were available in 85 patients. These showed an increase in exposure over time for all patients: week 1 median MPA trough was .9 μg/mL and week 6 median was 1.3 μg/mL. Two patient groups had consistently lower levels in the first 6 weeks after transplantation: children <16 years of age, whose levels ranged .3 to 1.2 μg/mL lower than adults, and recipients of myeloablative conditioning, whose levels ranged .4 to .8 μg/mL lower than those who received nonmyeloablative conditioning.

Based on the known importance of therapeutic early post-transplantation week CNI levels [21], we analyzed day 100 aGVHD risk according to the mean week 1 and 2 MPA trough levels using a level ≥ .5 μg/mL. We chose this concentration because a mean week 1 and 2 trough > .5 μg/mL is clinically significant for efficacy of aGVHD therapy [8]. Eighty-three patients had complete blood samples in the first 2 weeks after transplantation. Clinical factors such as age, gender, above or below 120% of ideal body weight, concomitant CNI, and albumin and bilirubin levels above or below the upper limit of normal were not associated with trough levels of <.5 or ≥.5 μg/mL, and CNI levels were therapeutic for all patients in the first 2 weeks (data not shown). Fifty-six of 69 (81%) patients who received MMF every 8 hours had trough levels ≥ .5 μg/mL compared with 8 of 14 (57%) in the 12-hour dosing group ($P = .078$).

There was no difference in the cumulative incidence of day 100 grade II to IV aGVHD in MPA trough level groups of <.5 and ≥.5 μg/mL (Figure 1A). Patients with troughs of <.5

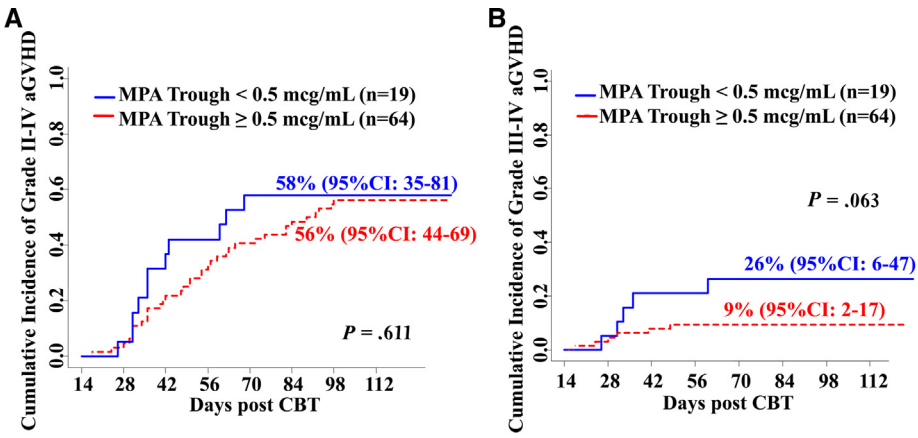

Figure 1. (A) Cumulative incidence of day 100 II to IV aGVHD by MPA trough level (n = 83). In a day 14 landmark analysis, mean week 1 and 2 MPA trough levels ≥ .5 μg/mL had no association with grades II to IV aGVHD by day 100. (B) Cumulative incidence of day 100 III and IV aGVHD by MPA trough level (n = 83). In a day 14 landmark analysis, the incidence of grade III and IV aGVHD at day 100 was lower in dCBT recipients with mean week 1 and 2 MPA trough levels ≥ .5 μg/mL.

μg/mL, however, had incidence of grade III and IV aGVHD of 26% versus 9% ($P = .063$) (Figure 1B). dCBT recipients (n = 10) who received a low total daily MMF dose (≤median of 43 mg/kg/day in this 83 patient subset) and had a low mean week 1 and 2 MPA trough level (<.5 μg/mL) had an incidence of grade III and IV aGVHD at day 100 of 40% (95% CI, 7% to 73%) as compared with that of 10% (95% CI, 3% to 16%) in the 73 patients with other dose and trough level combinations (ie, high MMF dose regardless of trough level or trough ≥ .5 μg/mL regardless of dose, $P = .008$).

To evaluate whether high MPA troughs correlated with myelosuppression or gastrointestinal toxicity, engraftment and duration of total parenteral nutrition (TPN) were analyzed. Concentrations of <2 and ≥2 μg/mL were chosen because ≥2 μg/mL have been associated with leukopenia in solid organ transplant recipients [22]. There was no difference in the cumulative incidence of day 100 neutrophil engraftment in myeloablated patients with mean week 1 to 2 MPA troughs of <2 and ≥2 μg/mL: 95% (95% CI, 89% to 100%) versus 100% (95% CI, 78% to 100%), $P = .422$. Also in myeloablated patients, there were no differences in platelet engraftment or duration of TPN (data not shown).

DISCUSSION

Although MMF immunosuppression in combination with a CNI has been used in CBT as aGVHD prophylaxis since the year 2000 [2], optimal dosing of MMF has not been established. In view of the significant incidence of aGVHD in our patient population [13], we have intensified MMF dosing to every 8 hours in dCBT recipients. However, in this analysis we analyzed transplantation outcomes according to the MMF dose in milligram per kilogram per day, as dosing has not accounted for recipient weight ≥ 50 kg, and dosing in small children has substantially changed over the time period of the study. Patients in each milligram per kilogram per day dosing groups were well matched, strengthening the multiple significant findings of this analysis. We found recipients who received a low milligram per kilogram per day dose of MMF had a significantly higher incidence of day 100 grade III and IV acute GVHD. Recipients of a low dose who had a highly HLA allele–mismatched dominant unit were at the worst risk. Finally, patients with mean week 1 and 2 MPA trough concentrations < .5 μg/mL had an increased day 100 grade III and IV aGVHD with no effect on hematopoietic recovery or gastrointestinal toxicity, as measured by TPN use. To our knowledge, this is the first large and comprehensive analysis to address the issue of MMF dosing in milligram per kilogram per day and include assessment of the clinical significance of MPA trough levels early after transplantation in CBT patients.

Important in this analysis is that when combining the MMF total daily dose with the degree of dominant unit-recipient HLA match, we noted that a higher MMF dose could potentially reduce the detrimental impact of having a lesser HLA-matched engrafting unit. This is clinically relevant, given our center's prior report that found a less well-matched dominant unit was associated with a greater grade III and IV aGVHD risk [13]. Although our findings must be confirmed in larger studies, they suggest that a higher MMF dose is beneficial in recipients of grafts with a high degree of HLA mismatch.

Although it is established that small children metabolize MMF more rapidly than adults, we did not identify a significant association between age and grade III and IV aGVHD (Tables 3 and 4). This may have been due to partial abrogation of aGVHD by the substantial increase in MMF dose in small children during the study period, and it is an important question for further investigation. An unexpected observation in this series was that male patients had a significantly higher incidence of grade III and IV aGVHD than women did. This was not due to an increased aGVHD risk in those with heavier weight or due to a greater risk in male patients engrafting with female units. This observation concerning gender is of great interest and remains unexplained.

The lack of difference in TRM between the low and high MMF dosing groups may have been due to aggressive supportive care administered to severe aGVHD cases. Additionally, as one half of the 22 GVHD-related deaths were after day 180, this diluted the sample size to be able to identify differences at day 180. This question of whether augmented MMF dosing can reduce GVHD-related TRM is critically important, however, and requires further investigation in a larger study. Nonetheless, it is clear that the GVHD deaths in this report were related to aGVHD, and in nearly all of these patients the GVHD began before day 100. Therefore, aGVHD is the correct target for prevention, and as severe aGVHD increases patient morbidity and transplantation costs, it is a serious complication to abrogate. Importantly, in relation to other survival endpoints, increased MMF dosing was not associated with an increase in deaths from infections or the incidence of relapse.

Previous reports support the efficacy of PK monitoring. Studies suggest that higher MPA exposure from an increased trough [23], steady-state concentration [24], or area under the curve [25,26] results in a lower aGHVD incidence although the ideal monitoring parameter has not been established. In this study, a mean week 1 and 2 MPA trough ≥ .5 μg/mL was hypothesized as clinically relevant for prophylaxis given its relationship with therapeutic efficacy [8]. We found that troughs < .5 μg/mL were associated with an increased grade III and IV aGVHD risk, complementing the findings of the larger 174 patient analysis. Moreover, that dCBT recipients who received a low total daily MMF dose/kg and had a low mean week 1 and 2 MPA trough had a very high incidence of grade III and IV aGVHD demonstrates that these patients, in particular, require increased MPA exposure. Although we did not identify an age effect in the 174 patient analysis, young children are potentially at special risk, given that patients < 16 years of age had lower troughs in our analysis over the 6-week period, as expected given their faster MMF metabolism [27]. Indeed, Osunkwo et al. have proposed an increased MMF dosing to 900 to 1200 mg/m^2 every 6 hours to achieve levels ≥ 1 μg/mL, which corresponds to nearly 30 mg/kg/dose in children [23].

From the stand point of engraftment, an increased total daily MMF dose per kilogram had no deleterious effect. For this MPA trough level toxicity analysis, we choose a 2 μg/mL cut-off based on solid organ transplantation literature [22]. The lack of any association between 1 to 2 week troughs and engraftment supports the findings of the larger 174 patient analysis. The upper threshold for total MPA troughs in CBT has yet to be established, however, and the possible toxicity of very high levels could remain a clinical concern. Although Sanz et al. reported a delay in neutrophil engraftment with the use of MMF compared with corticosteroids in patients receiving single CBT [3], we have no evidence to indicate that intensified dosing as investigated in this MSKCC analysis is detrimental in dCBT. A potential risk of gastrointestinal toxicity was also not substantiated, and there were no cases of MMF colitis.

Multiple questions concerning MMF PK remain unanswered. Though the majority of our patients remained on i.v. MMF for the duration of the PK subset period, the impact of changing from i.v. to oral drug was not addressed in this analysis and could be important given the potential drop in bioavailability [28]. Additionally, whether intensified MMF dosing later in the transplantation course can exacerbate myelosuppression is possible, especially in the setting of other myelosuppressive drugs, such as valganciclovir. Finally, how to correctly taper MMF after transplantation is not established. All of these questions require formal future investigation.

This analysis supports intensified MMF dosing based on the total daily dose per kilogram, even in adult dCBT recipients receiving relatively low unit cell doses. As a recognized adult dosage is 15 mg/kg/dose for patients < 50 kg, we propose patients ≥ 50 kg also receive 15 mg/kg/dose every 8 hours with a dose cap of 1.5 gm every 8 hours. This intervention could prove especially important in heavy patients and/or recipients of highly HLA-mismatched units. Furthermore, although the ideal dosing in children less than 12 years remains unknown, we now support intensified dosing of 30 mg/kg/dose (nearly 900 mg/m^2/dose) as previously suggested in Bhatia et al. [29] to optimize MPA exposure in this population. Finally, obtaining MPA trough levels (ideally with rapid result availability) during the first 2 weeks after transplantation to target a trough level $\geq .5$ μg/mL is also appropriate in dCBT recipients.

ACKNOWLEDGMENTS

Financial disclosure: This work was supported in part by the Gabrielle's Angel Foundation for Cancer Research (J.N.B.), the Memorial Sloan-Kettering Cancer Center Society (J.N.B.), the Translational and Integrative Medicine Research Grant (J.N.B.), P01 CA23766 from the National Cancer Institute, National Institutes of Health (J.N.B.), the Society of Memorial Sloan Kettering Cancer Center (J.N.B.), and the New York State Empire Clinical Research Investigator Program (D.M.P.).

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: S.H., D.M.P., and J.N.B. designed the study, interpreted the data, and wrote the manuscript. P.H., J.Z., and S.M.D. analyzed the data and wrote the manuscript. M.L. analyzed the data. M.P., S.M., N.A., N.A.K., R.O., S.P., A.S., A.H., R.J., M.M.v.B., S.G., M.A.P., and J.W.Y. wrote the manuscript.

REFERENCES

1. Bolwell B, Sobecks R, Pohlman B, et al. A prospective randomized trial comparing cyclosporine and short course methotrexate with cyclosporine and mycophenolate mofetil for GVHD prophylaxis in myeloablative allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 2004;34:621-625.
2. Barker JN, Weisdorf DJ, DeFor TE, et al. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. *Blood*. 2005;105:1343-1347.
3. Sanz J, Picardi A, Hernandez Boluda JC, et al. Impact of graft-versus-host disease prophylaxis on outcomes after myeloablative single-unit umbilical cord blood transplantation. *Biol Blood Marrow Transplant*. 2013;19:1387-1392.
4. Okamura A, Shimoyama M, Ishii S, et al. Delayed neutrophil engraftment in cord blood transplantation with intensive administration of mycophenolate mofetil for GVHD prophylaxis. *Bone Marrow Transplant*. 2011;46:148-149.
5. Kuypers DR, Le Meur Y, Cantarovich M, et al. Consensus report on therapeutic drug monitoring of mycophenolic acid in solid organ transplantation. *Clin J Am Soc Nephrol*. 2010;5:341-358.
6. Jacobson P, Miftek J, Rogosheske J, et al. Highly variable mycophenolate pharmacokinetics in hematopoietic stem cell transplantation (HSCT): potential need for clinical drug monitoring. *Blood*. 2002;100:411a.
7. Jacobson P, Rogosheske J, Barker JN, et al. Relationship of mycophenolic acid exposure to clinical outcome after hematopoietic cell transplantation. *Clin Pharmacol Ther*. 2005;78:486-500.
8. Jacobson PA, Huang J, Wu J, et al. Mycophenolate pharmacokinetics and association with response to acute graft-versus-host disease treatment from the Blood and Marrow Transplant Clinical Trials Network. *Biol Blood Marrow Transplant*. 2010;16:421-429.
9. Giaccone L, McCune JS, Maris MB, et al. Pharmacodynamics of mycophenolate mofetil after nonmyeloablative conditioning and unrelated donor hematopoietic cell transplantation. *Blood*. 2005;106:4381-4388.
10. Maris MB, Sandmaier BM, Storer BE, et al. Unrelated donor granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cell transplantation after nonmyeloablative conditioning: the effect of postgrafting mycophenolate mofetil dosing. *Biol Blood Marrow Transplant*. 2006;12:454-465.
11. Baron F, Maris MB, Storer BE, et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with chronic myeloid leukemia. *Biol Blood Marrow Transplant*. 2005;11:272-279.
12. MacMillan ML, Weisdorf DJ, Brunstein CG, et al. Acute graft-versus-host disease after unrelated donor umbilical cord blood transplantation: analysis of risk factors. *Blood*. 2009;113:2410-2415.
13. Ponce DM, Gonzales A, Lubin M, et al. Graft-versus-host disease after double-unit cord blood transplantation has unique features and an association with engrafting unit-to-recipient HLA match. *Biol Blood Marrow Transplant*. 2013;19:904-911.
14. Brunstein CG, Gutman JA, Weisdorf DJ, et al. Allogeneic hematopoietic cell transplantation for hematologic malignancy: relative risks and benefits of double umbilical cord blood. *Blood*. 2010;116:4693-4699.
15. Jacobson P, El-Massah SF, Rogosheske J, et al. Comparison of two mycophenolate mofetil dosing regimens after hematopoietic cell transplantation. *Bone Marrow Transplant*. 2009;44:113-120.
16. Li H, Mager DE, Sandmaier BM, et al. Population pharmacokinetics and dose optimization of mycophenolic acid in HCT recipients receiving oral mycophenolate mofetil. *J Clin Pharmacol*. 2013;53:393-402.
17. Barker JN, Byam C, Scaradavou A. How I treat: the selection and acquisition of unrelated cord blood grafts. *Blood*. 2011;117:2332-2339.
18. Ponce DM, Sauter C, Devlin S, et al. A novel reduced-intensity conditioning regimen induces a high incidence of sustained donor-derived neutrophil and platelet engraftment after double-unit cord blood transplantation. *Biol Blood Marrow Transplant*. 2013;19:799-803.
19. Rowlings PA, Przepiorka D, Klein JP, et al. IBMTR Severity Index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade. *Br J Haematol*. 1997;97:855-864.
20. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant*. 2005;11:945-956.
21. Malard F, Szydlo RM, Brissot E, et al. Impact of cyclosporine-A concentration on the incidence of severe acute graft-versus-host disease after allogeneic stem cell transplantation. *Biol Blood Marrow Transplant*. 2010;16:28-34.
22. Hao C, Anwei M, Bing C, et al. Monitoring mycophenolic acid pharmacokinetic parameters in liver transplant recipients: prediction of occurrence of leukopenia. *Liver Transpl*. 2008;14:1165-1173.
23. Osunkwo I, Bessmertny O, Harrison L, et al. A pilot study of tacrolimus and mycophenolate mofetil graft-versus-host disease prophylaxis in childhood and adolescent allogeneic stem cell transplant recipients. *Biol Blood Marrow Transplant*. 2004;10:246-258.
24. McDermott CL, Sandmaier BM, Storer B, et al. Nonrelapse mortality and mycophenolic acid exposure in nonmyeloablative hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2013;19:1159-1166.
25. Okamura A, Yamamori M, Shimoyama M, et al. Pharmacokinetics-based optimal dose-exploration of mycophenolate mofetil in allogeneic hematopoietic stem cell transplantation. *Int J Hematol*. 2008;88:104-110.
26. Royer B, Larosa F, Legrand F, et al. Pharmacokinetics of mycophenolic acid administered 3 times daily after hematopoietic stem cell transplantation with reduced-intensity regimen. *Biol Blood Marrow Transplant*. 2009;15:1134-1139.
27. Kearns GL, Abdel-Rahman SM, Alander SW, et al. Developmental pharmacology—drug disposition, action, and therapy in infants and children. *N Engl J Med*. 2003;349:1157-1167.
28. Frymoyer A, Verotta D, Jacobson P, Long-Boyle J. Population pharmacokinetics of unbound mycophenolic acid in adult allogeneic haematopoietic cell transplantation: effect of pharmacogenetic factors. *Br J Clin Pharmacol*. 2013;75:463-475.
29. Bhatia M, Militano O, Jin Z, et al. An age-dependent pharmacokinetic study of intravenous and oral mycophenolate mofetil in combination with tacrolimus for GVHD prophylaxis in pediatric allogeneic stem cell transplantation recipients. *Biol Blood Marrow Transplant*. 2010;16:333-343.